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Assessment of Pod Coats for the Study of Antioxidant Prospective in Cowpea, Mungbean and Moth Bean

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A B S T R A C T

Cereals and pulses are the rich sources of protein and energy and thus hold an important place in human diet mainly in developing countries. In present study, pod coats of locally grown pulse crops (cowpea, mungbean and mothbean) were tested for various phytochemical constituents and antioxidant activity. Pod coats of these pulse crops were extracted with three solvents (acetone, ethylacetate and chloroform) and tested for total phenols, flavonoids, tannins, tocopherols and DPPH free radical scavenging activity. Highest extract yield was observed in acetone extract of mothbean (4.45%) while lowest in chloroform extract of cowpea (2.99%). Total phenolics content ranged between 7.24 mg $GAEg^{-1}$ (chloroform extract of cowpea) to 15.59 mg $GAEg^{-1}$ (acetone extract of mothbean). Flavonoid content of pod coats of these pulses varied from 3.30 mg $CAEg^{-1}$ (chloroform extract of cowpea) to 6.05 mg $CAEg^{-1}$ (acetone extract of mothbean). Tocopherols content was highest in ethylacetate extract of mungbean (5.76 mg/g of extract) while lowest in chloroform extract of cowpea (3.41 mg/g of extract). Tannin content was highest in acetone extract of mothbean (1.86 mg $TAEg^{-1}$) while lowest in chloroform extract of mungbean (0.71 mg $TAEg^{-1}$). Antioxidant activity was determined by DPPH method. Acetone extract of mothbean exhibited highest DPPH free radical scavenging activity (74.21%) while it was lowest in chloroform extract of cowpea (54.95%) at a concentration of 1.0 mg/mL of the extract. The results of the present analytical study revealed that pod coat of these three pulse crops may be a valuable source of natural antioxidants and are potentially applicable in food and medicinal industry

Introduction

Legumes, the staple food in many regions of the world recently have been studied for their antioxidant potential, because of increasing interest about the health benefits associated with antioxidants. Legumes are an economic source of proteins, dietary fibres, micronutrients and other bioactive compounds like polyphenols. Besides the nutritional value, legumes have therapeutic properties and promote good health (Geil and Anderson, 1994). The leaves, tender stems and pod coats of legumes are left behind as byproducts which can be used as fairly nutritious cattle feed. They are rich sources of digestible protein. There is a paucity of information regarding the health promoting components in

these agricultural wastes specifically phenolic compounds which regulate oxidative stress and influences the enzyme activities linked to hypertension and hyperglycemia. This information may assist to exploit the use of agri-waste as a tool in reducing disease and promoting overall health.

Cowpea (*Vigna unguiculata* L.) is one of the most important tropical legumes of the family Fabaceae. It was grown in Africa, but these days it is also cultivated in Southern United States and Southeast Asia. It is an annual herb which grows in adequate rainfall and warm climate. The immature pods and young leaves are eaten as vegetables. Threshed pod husk of cowpea is also used to feed livestock. It is known to have high fibre content and low fat which helps to prevent heart disease by lowering the low-density lipoproteins [Phillips *et al.*, 2007]. Seeds and leaves are used as a bandage to treat insect stings, skin swellings and infections. Mungbean (*Vigna radiata* L.) alternatively known as green gram. It is a short season legume cultivated widely in summers throughout the tropics and subtropics. It forms an important part of cereal-based diet in many countries like Thailand, Pakistan, India, China, Indonesia and Philippines [Jansen *et al.*, 1996]. It is a rich source of protein, fibre, antioxidants and various vitamins. It is very popular for its low flatulence and better digestibility. Due to their detoxification properties, they are used to alleviate heat stroke, reduce swelling and refresh mentality. This pulse can work as a cover crop in-between cereal crop. It makes good green manure.

Mothbean (*Vigna aconitifolia* L.) belongs to family Fabaceae, is an exceptionally hard legume thrives in hot, dry, tropical conditions of South Asia. Mothbean is also known by various other names including math, mat, matki, or moth bean. Being a rich source of protein and other elements, seed and sprouts of this plant are an exceptionally good supplement to cereal-based diets. Mothbean contains nearly 34-40% starch which is used for the preparation of noodles in the food industry. In rural areas of India, the seeds of mothbean are commonly consumed after processing like dry heating or soaking followed by cooking, along with cooked sorghum, rice or pearl millet. With the current upsurge of interest in substitution of synthetic antioxidants by natural ones and efficiency of natural antioxidants in biological system has fostered research on screening of agricultural waste for finding new antioxidants. Hence the present study was conducted to determine total phenols, flavonoids, tocopherols, tannins and antioxidant activities of pod coats of cowpea, mungbean and mothbean.

Materials and Methods

Preparation of extracts

The threshed pods of cowpea, mungbean and mothbean were collected from the experimental field of CCS HAU Hisar, Haryana. Threshed pods were sorted manually to separate pod coats from stones, grass residue and other plant parts. The pod coats of all the three pulse crops were ground into powder by using an electric grinder. The powdered samples were extracted with petroleum ether (60-80°C). 100g dried defatted powdered sample of each crop were then extracted separately by the soxhlet method using acetone, ethyl acetate and chloroform for 8h. Extracts were used for quantitative analysis of total phenols, flavonoids, tocopherols, tannins and DPPH free radical scavenging activity.

Determination of total phenolic content

The total phenolics were determined by using Folin-Ciocalteu method using gallic acid as standard (Singleton and Rossi, 1965). For

estimation of total phenolics in various extracts of pod coats of pigeonpea, added 1.0 mL of Folin-Ciocalteu reagent and 2.0 mL of Na_2CO_3 (20%, w/v) to the 1mL extract, mixed and final volume was made up to 50.0 mL with water.

The mixture was kept undisturbed for 50 min. and then centrifuged at 6000 rpm for 10 minutes. Supernatant was removed and its absorbance was measured at 730 nm and total phenolic content was expressed as mg of gallic acid equivalent per g (mg $GAEg^{-1}$).

Determination of flavonoids

The aluminium chloride colorimetric method was used for estimation of flavonoids [Zhishen *et al.,* 1999]. One mL of diluted extract was taken in a test tube. To the extract, 5% $NaNO_2$ (0.3 mL) was added. After 5 minutes 10% $AlCl_3$ (0.3 mL) was added. Immediately, 1M NaOH (2 mL) was added and content was mixed and diluted with distilled water to final volume 10 mL.

The absorbance was taken at 510 nm against blank. Flavonoids content of samples was expressed as mg catechin equivalent per g of the extract (mg $CAEg^{-1}$).

Determination of tocopherol content in various pod coat extracts of different pulse crops

Total tocopherols were estimated by Philip's method using α-tocopherol as standard [Pearson, 1976]. Aliquots of 10, 20, 30, 40, 50 and 60 ppm solution of α-tocopherol in ethanol and extract were transferred to a flask and volume was made to 8mL with ethanol. 1mL 2,2- dipyridyl reagent was added to this mixture, followed by 1mL $FeCl_3.6H_2O$ reagent. Mixture was shaken for 10 seconds and then absorbance was taken at 520 nm against ethanol as blank.

Determination of tannin content in various pod coat extracts of different pulse crops

Tannin content was determined by Pearson method with slight modification using tannic acid as standard (Philip *et al.,* 1954). One mL of extract or standard tannic acid solution (0.01, 0.02, 0.03, 0.04, 0.05, and 0.06 mg/mL) was taken in different test tubes and 1mL of Folin – Denis reagent was added to each test tube followed by 2.5 mL of saturated sodium carbonate solution. Total volume was made to 10 mL with distilled water and shaken to mix properly. Thereafter mixtures were incubated at room temperature (approximately 30ºC) for 30 minutes and absorbance was measured against the reagent blank at 760 nm wavelength.

2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant activity of the extracts was evaluated by DPPH free radical scavenging method (Hatano *et al.,* 1988). Briefly 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 mg of extract were mixed separately with 2.5 mL of 2,2'-diphenyl-2-picrylhydrazyl radical (DPPH: $0.025gL^{-1}$ in methanol) and final volume was made to 10 mL with methanol and mixed by vortex for 5 minute and then immediately placed in spectrophotometer Spectronic 20 (Milton Roy Company).

The absorbance was measured after every 10 min. at 517 nm, as the reaction reached at plateau (time at steady state) gradually. The antioxidant activity was expressed as the percentage of decline of the absorbance after 2 hours, relative to the control, corresponding to the percentage of DPPH scavenged.

Calculation

The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\% \text{ DPPH } *_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.

Results and Discussion

Estimation of yield of pod coat extracts of various pulses under study

The yield of extract in the three solvents varied slightly. Extract yield of acetone, ethylacetate and chloroform extracts of cowpea were 3.69, 3.36 and 2.99 g/100g, respectively. In cowpea, highest yield was observed in acetone extract. In case of mungbean, there is not much difference in extract yield of different solvents. Acetone extract had the highest yield (3.71) followed by ethylacetate extract (3.45) and chloroform extract (3.04) in g/100g pod coat. Similarly mothbean also displayed highest yield in acetone extract (4.45) followed by ethylacetate extract (3.85) and chloroform extract (3.15). It was observed that among three solvents, acetone had highest extract yield while chloroform had lowest extract yield in all three pulse crops. Among three crops moth bean had highest yield in all three solvents. The difference in the yield with different solvent is attributed to polarity of different compounds present in pod coats (Jayaprakash *et al.*, 2001). But the low yield of chloroform extract might be due to the presence of smaller amount of methylated derivatives of various phenols, terpenoids and sterols in pod coats.

Estimation of total phenolics in pod coat extracts of different pulse crops

Analysis of total phenolics in pod coat extracts of cowpea showed that, acetone extract holds the highest amount of total phenols i.e. 10.11 mg $GAEg^{-1}$ while ethylacetate (8.15 mg $GAEg^{-1}$) and chloroform extract (7.24 mg $GAEg^{-1}$) showed a comparable amount of total phenolics (Table 1). Similarly in pod coat extracts of mungbean, acetone extract showed highest phenolic content i.e. 10.75 mg $GAEg^{-1}$ followed by ethylacetate extract (9.51 mg $GAEg^{-1}$) and chloroform extract (8.57 mg $GAEg^{-1}$) (Table 2). In case of mothbean as well, highest phenolic content was observed in acetone extract i.e. 15.59 mg $GAEg^{-1}$ followed by ethyl acetate extract (13.56 mg $GAEg^{-1}$) and chloroform extract (10.54 mg $GAEg^{-1}$) (Table 3).

Estimation of flavonoids in pod coat extracts of different pulse crops

Flavones, isoflavones and flavonols are the subgroups of flavonoids. In cowpea extracts, flavonoid content (mg $CAEg^{-1}$) was highest in acetone extract (4.54) followed by ethylacetate extract (3.48) and chloroform extract (3.30) (Table 1). In mungbean pod coat extracts, flavonoid content in acetone, ethylacetate and chloroform extract were 5.12, 4.16 and 3.63 mg $CAEg^{-1}$ respectively (Table 2). Similarly in mothbean pod coat extracts, highest flavonoid content (mg $CAEg^{-1}$) was observed in acetone extract (6.05) followed by ethylacetate extract (5.16 mg) and chloroform extract (4.33) (Table 3).

Estimation of tocopherols in pod coat extracts of different pulse crops

Tocopherols are methylated phenols many of which have vitamin E activity. Ethylacetate extract cowpea had maximum tocopherol content (3.78 mg/g of extract) while acetone (3.42 mg/g of extract) and chloroform (3.41 mg/g of extract) extracts have almost equal amount of tocopherols. Tocopherol content of different extracts of mungbean pod coat is shown in Table 2. Ethylacetate extract showed highest tocopherol content (5.76 mg/g of extract) followed by acetone extract (5.14 mg/g of extract) and chloroform extract (4.70 mg/g of extract).

Table.1 Chemical composition of pod coat extracts of cowpea

S. No	Phytochemical constituents	Acetone Extract	Ethylacetate extract	Chloroform extract
1.	Total Phenolics (mg GAE/g)	10.11±0.09	8.15±0.11	7.24±0.08
2.	Flavonoids (mg CAE/g)	4.54±0.06	3.48±0.03	3.30±0.04
3.	Tocopherols (mg/g extract)	3.42±0.05	3.78±0.04	3.41±0.02
4.	Tannin content (mg TAE/g)	1.55±0.13	1.23±0.05	1.17±0.08

Values are mean of three replicates ± standard error

Table.2 Chemical composition of pod coat extracts of mungbean

S. No	Phytochemical constituents	Acetone Extract	Ethylacetate extract	Chloroform extract
1.	Total Phenolics (mg GAE/g)	10.75±0.19	9.51±0.09	8.57±0.06
2.	Flavonoids (mg CAE/g)	5.12±0.03	4.16±0.08	3.63±0.05
3.	Tocopherols (mg/g extract)	5.14±0.06	5.76±0.09	4.70±0.04
4.	Tannin content (mg TAE/g)	1.10±0.02	0.93±0.03	0.71±0.03

Values are mean of three replicates ± standard error

Table.3 Chemical composition of pod coat extracts of mothbean

S. No	Phytochemical constituents	Acetone Extract	Ethylacetate extract	Chloroform Extract
1.	Total Phenolics (mg GAE/g)	15.59±0.14	13.56±0.12	10.54±0.04
2.	Flavonoids (mg CAE/g)	6.05±0.05	5.16±0.04	4.33±0.05
3.	Tocopherols (mg/g extract)	4.39±0.07	5.70±0.09	3.80±0.05
4.	Tannin content (mg TAE/g)	1.86±0.04	1.59±0.05	1.28±0.04

Values are mean of three replicates ± standard error

Fig.1 DPPH free radical scavenging activity (%) of BHA (standard) and pod coat extracts of cowpea

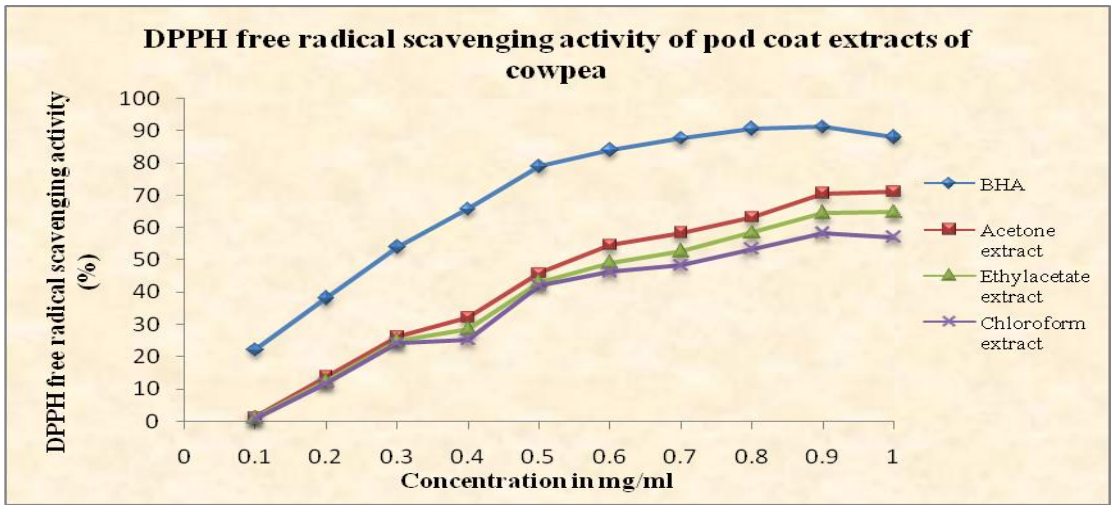


Fig.2 DPPH free radical scavenging activity (%) of BHA (standard) and Pod coat extracts of mungbean

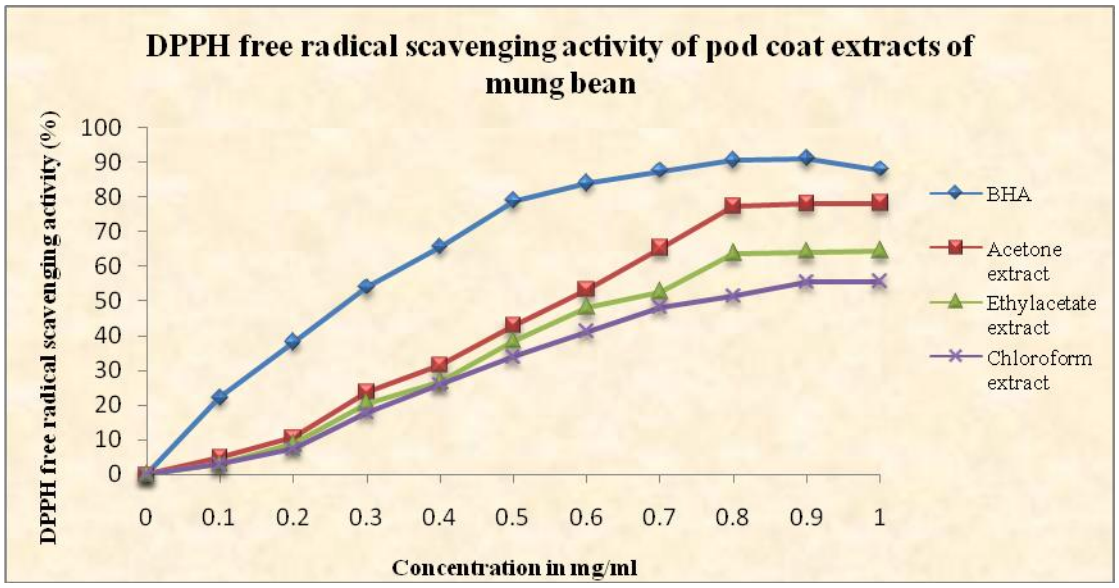
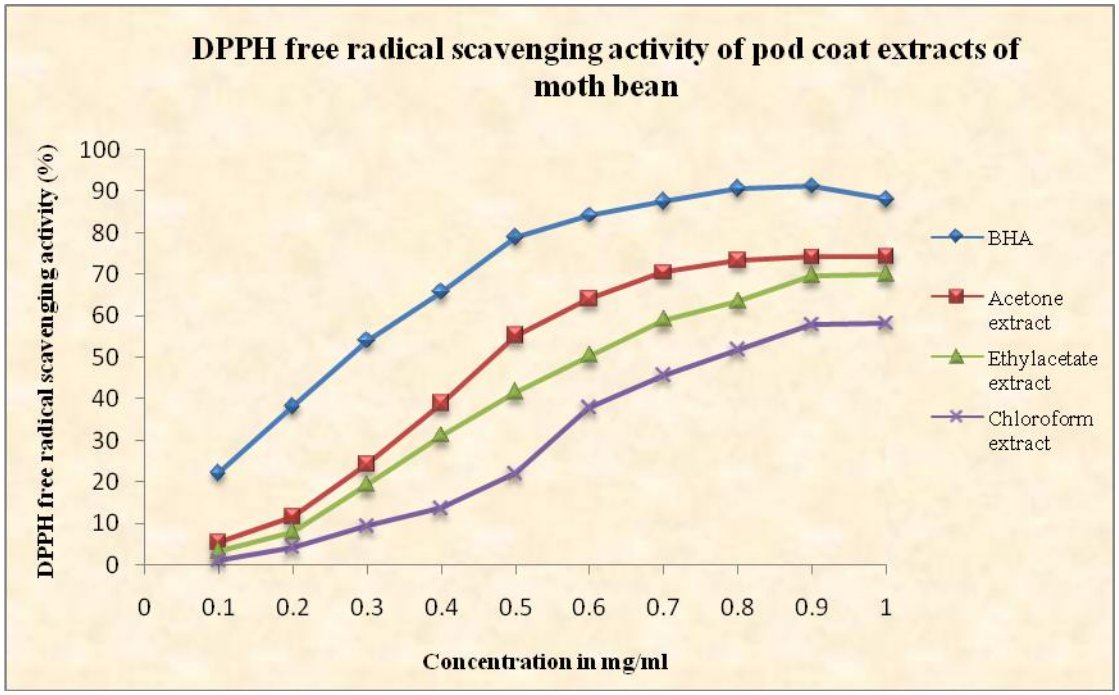


Fig.3 DPPH free radical scavenging activity (%) of BHA (standard) and Pod coat extracts of mothbean



Tocopherol content of different extracts of mothbean pod coat is shown in Table 3. Ethylacetate extract showed highest tocopherol content (5.70 mg/g of extract) followed by acetone extract (4.39 mg/g of extract) and chloroform extract (3.80 mg/g of extract). Tocopherol contents in mothbean extracts were lower than corresponding

extracts of mungbean but higher than that of cowpea.

Estimation of tannins in pod coat extracts of different pulse crops

Analysis of tannin content showed that (Table 2), acetone extract of cowpea contained highest amount of tannins (1.55 mg $TAEg^{-1}$) followed by ethylacetate (1.23 mg $TAEg^{-1}$) and chloroform extract (1.17 mg $TAEg^{-1}$). In case of pod coat extracts of mungbean, acetone extract contained highest amount of tannins (1.10 mg $TAEg^{-1}$) followed by ethylacetate (0.93 mg $TAEg^{-1}$) and chloroform extract (0.71 mg $TAEg^{-1}$). In mothbean extracts, highest tannin content was found in acetone (1.86 mg $TAEg^{-1}$) followed by ethylacetate (1.59 mg $TAEg^{-1}$) and chloroform extract (1.28 mg $TAEg^{-1}$). It was noted that mothbean extracts had highest tannin content while mungbean had lowest among three pulse crops.

Evaluation of antioxidant activities of pod coat extract of different pulse crops

Antioxidant activities were evaluated by 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) method. In this method BHA was used as standard for measuring DPPH free radical scavenging activity. IC_{50} value obtained for BHA solution was 0.28 mg/mL. Maximum DPPH free radical scavenging activity exhibited by BHA was 91.14% observed at 0.9 mg/mL concentration. Graphical representation for (%) antioxidant activity in BHA as well as different extracts of cowpea by DPPH method is displayed in Figure 1. The maximum antioxidant activities exhibited by acetone extract, ethylacetate extract and chloroform extract of cowpea were 71.09%, 64.81% and 54.95% respectively, at 1.0 mg/mL concentration of the extract. The corresponding IC_{50} values to scavenge $DPPH^{\bullet}$ radical were 0.57, 0.63, and 0.71 mg/mL of the extract. Acetone extract showed highest free radical scavenging activity.

The maximum antioxidant activities exhibited by acetone extract, ethylacetate extract and chloroform extract of mungbean were 72.16%, 64.54% and 55.56% respectively, at the concentration of 1.0 mg/mL of the extract. The corresponding IC_{50} values to scavenge $DPPH^{\bullet}$ radical were 0.55, 0.65, and 0.78 mg/mL of the extract. Here also, acetone extract showed highest free radical scavenging activity (Fig. 1–3).

The maximum antioxidant activities exhibited by acetone extract, ethylacetate extract and chloroform extract of mothbean were 74.21%, 70.03% and 58.14% respectively, at the concentration of 1.0 mg/mL of the extract. The corresponding IC_{50} values to scavenge $DPPH^{\bullet}$ radical were 0.47, 0.61, and 0.83 mg/mL of the extract. Acetone extract exhibited highest free radical scavenging activity. Among three crops highest free radical scavenging activity was observed in pod coat extracts of mothbean while it was comparable in pod coat extracts cowpea and mungbean at a concentration of 1mg/ mL of extract. Our results clearly indicates a positive correlation between phenolic composition and antioxidant activity, as the higher free radical scavenging activity of acetone extracts could be due to higher phenolics and flavonoids content.

We can conclude from the results in the present investigation that pod coat of cowpea, mungbean and mothbean are rich in phenols, flavonoids and tocopherols. Our results provided evidence that acetone extract of mothbean pod coat may provide potential natural antioxidants for the food industry and other fields. However, further studies are urgently needed for screening of the active components with enhanced antioxidant properties in pod coats of these pulse crops.

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